Immunological parameters as a new lead in the diagnosis of ovarian cancer

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Abstract

Ovarian cancer is the leading cause of pelvic gynecological cancer death in Europe. Prognosis is poor in women diagnosed at stage III to IV or in case disease recurs. Platin-based chemotherapy and radical surgery have already improved prognosis significantly. Novel strategies in the treatment of ovarian cancer are being searched for. The study of the immune system as a valuable parameter in the development of ovarian cancer has been neglected for a long time. Nevertheless, this is a field in full progression and might open new perspectives in the diagnosis and prognosis of ovarian cancer patients and could lead to a more motivated choice of targeted therapies.

Key words: **Ovarian cancer, diagnosis, prognostic marker, immunosuppression, regulatory T cell, myeloid derived suppressor cells, tumor-associated macrophages.**

Introduction

Ovarian cancer is the second most important type of pelvic tumor in women. Worldwide up to 240 000 women are diagnosed with ovarian cancer each year (WHO, 2014). As signs and symptoms in early ovarian cancer are scarce, up to 63% of patients are diagnosed at stage III or IV, leading to poor prognosis (Vergote et al., 2010). Until now, prognosis is based on the histological type, grade and the staging according to the FIGO classification.

Gynecological ultrasound has proven very useful and accurate in the diagnosis of ovarian cancer, though is subjective. Over the past decade, large progress has been made to standardize and optimize the classification of adnexal masses by the International Ovarian Tumour Analysis (IOTA) group, by means of logistic regression models. Meta-analyses and external validation studies have shown superiority of the IOTA models (Van Calster et al., 2014). In contrast, no serum marker has shown to be specific enough in the diagnosis of adnexal masses as a standalone. However, the most recent model of the IOTA group, the ADNEX model, combines ultrasound characteristic, clinical data and a serum marker, CA125 (Table I). (Van Calster et al., 2014). Whole body Magnetic Resonance Imaging (MRI) with diffusion weighting is an advantage in the preoperative staging of patients with ovarian cancer, providing important information about retroperitoneal and thoracic nodes (Michielsen et al., 2014). However in the diagnosis of difficult to classify adnexal masses the advantage of MRI compared to transvaginal ultrasound by experts is limited (Kaijser et al., 2014).

So far, research on prognostic markers has focused on genetic markers influencing survival and treatment response in ovarian cancer. In 1996 the first reports were published that suggested that BRCA1/2 germline mutation is correlated with improved outcome (Rubin et al., 1996). This was underscored by a recent systematic review (Sun et al., 2014). Recently, a generalized genetic mapping of high-grade serous ovarian cancer was performed by the Cancer Genome Atlas Network (TCGA). They created 4 subtypes based on mutation analysis,

Table I. — Criteria used in the IOTA ADNEX model.

Age of the patient at examination	Years
Referral center for Gynecological Oncology	Yes/No
Maximal diameter of the lesion	mm
Maximal diameter of the largest solid part	mm
More than 10 locules	Yes/No
Number of papillary projections	None One/Two/Three More than three
Acoustic shadow present	Yes/No
Ascites (outside the pelvis)	Yes/No
Serum CA-125	U/mL

mRNA and miRNA expression and DNA methylation: immunoreactive, differentiated, proliferative and mesenchymal. T-cell chemokine ligands CXCL11 and CXCL10 and the receptor CXCR3 characterized the immunoreactive subtype (The Cancer Genome Atlas Research Network, 2011). This article highlights the importance of the immune system in ovarian cancer. Indeed, once malignant cells arise, the immune system will control their development mainly by the presence of effector T cells (Teff). This stage is called the immune surveillance. If the operation is successful, the malignant cells are eliminated or reside in a dormant state (elimination vs equilibrium). However, cells might also escape the control of the immune system, start proliferating and the tumor becomes clinically apparent (Schreiber et al., 2011). The tumor develops several strategies to evade the immune system. First and most important is the attraction of immunosuppressive cells into the tumor. In the tumor microenvironment there is a rise in immunosuppressive cells and molecules that render the Teff less functional. Most important players seem to be regulatory T cells (Treg), myeloid derived suppressor cells (MDSC) and tumor-associated macrophages (TAM). As a second line of defense the tumor inhibits the infiltration of Teff into the tumor through a decrease in chemotactic molecules and a decreased quality of the tumoral vascularization. Current research in onco-immunology is focusing on methods to overcome this immune-escape.

It is clear that the immune system is an important regulator in the development of cancer. It can be presumed that the determination of these immune cells might give rise to new diagnostic and even prognostic markers for ovarian cancer. Early research in colorectal cancer suggests that the presence of Teff in the tumor might be more important than traditional staging to predict outcome (Galon et al., 2006). In ovarian cancer it has been shown that a high ratio of Teff compared to immunosuppressive Treg in the tumor is associated with better prognosis (Zhang et al., 2003; Curiel et al., 2004). It has become clear that merely the presence of Teff is not enough, the balance between Teff and immunosuppressive cells is important. In a study in metastatic melanoma, Teff were isolated from the tumor microenvironment and analyzed. Immediately after isolation they showed a suboptimal cytokine production and proliferation, however this was normalized when these T cells were expanded *ex vivo* and stimulatory cytokines were added (Harlin et al., 2006). This underlines the importance of the immunosuppressive tumor microenvironment.

Immunosuppressive cells in general

Regulatory T cells (Treg) are a subpopulation of T cells, which maintain tolerance to self-antigens by influencing the activity of other Teff. Their physiological function is to prevent auto-immunity and down regulate undesired T cell responses. We can differentiate between 2 subsets of regulatory T cells. The naturally occurring Treg develop in the thymus and are responsible for central inhibition of auto-immunity. T cells that leave the bone marrow migrate to the thymus to mature. In the thymus autoreactive T cells are deleted. Adaptive Treg develop as a consequence of continued peripheral T cell activation, as is present in chronic inflammation and tumor development. Treg are trafficked to the tumor site through presence of chemokines such as CCL2 and CCL22 in the tumor microenvironment. Expansion of Tregs can be induced by tolerogenic dendritic cells (DCs) through the expression of indoleamine 2,3-dioxygenase (IDO). Transforming epidermal growth factor beta (TGF-β) can convert effector T cells into regulatory T cells leading to further increase immunosuppression (Gajewski et

al., 2013). Treg exert their immunosuppressive function through several mechanisms as depicted in Figure 1 (Zou, 2006). Treg will promote IDO expression and lead to depletion of tryptophan. This leads to T cell anergy and apoptosis of T cells. Furthermore stimulation of IDO will also stimulate a feedback loop by inducing tolerogenic DCs (Katz et al., 2008). Treg will also increase their expression of negative co-stimulatory molecules, such as Programmed Death-1 (PD-1), Programmed Death Ligand 1(PD-L1) and CTLA-4, leading to increased apoptosis in Teff (Ooi et al., 2014). Treg will also secrete IL-10 and TGF-β. IL-10 is an anti-inflammatory cytokine that will cause dysfunction of antigen presenting cells (APC) through decreased expression of MHC molecules and other co-stimulatory molecules and a decrease in circulating IL-12 (Maloy et al., 2001; Zheng et al., 2004). Secretion of TGF-β causes unresponsiveness in tumor infiltrating effector T cells through FoxP1 transcription factor expression (Zheng et al., 2004; Stephen et al., 2014). Parallel to IDO, TGF-β expression will stimulate an immunosuppressive feedback-loop to stimulate the transformation of Teff to Treg (Zou, 2006).

Myeloid derived suppressor cells (MDSCs) are a heterogeneous population of early myeloid progenitors at different stages of differentiation. These cells are capable of suppressing the innate (natural killer cells (NK)/non-specific defense) and the adaptive immune system (Teff/specific defense mechanisms). MDSC are influenced by pro-inflammatory cytokines and their presence in the tumor microenvironment could be one of the causes of tumor-associated immunosuppression. The presence of circulating MDSC correlates with poor prognosis, increased metastatic potential and tumor evasion of host immunity. As depicted in Figure 2, the accumulation of MDSC in the tumor micro-environment is stimulated by several factors such as: granulocyte macrophage colony stimulating factor (GM-CSF), vascular endothelial growth factor (VEGF), chemokines such as CCL-2 and TGF-β, produced by the tumor and the intratumoral immune cells (Nagaraj et al., 2010; Talmadge et al., 2013). MDSC will recruit and induce Treg through production of IL-10 and TGF-β and the expression of chemotactic molecules on the cell surface of the MDSC. MDSC are myeloid lineage cells and can differentiate into DC when matured; however

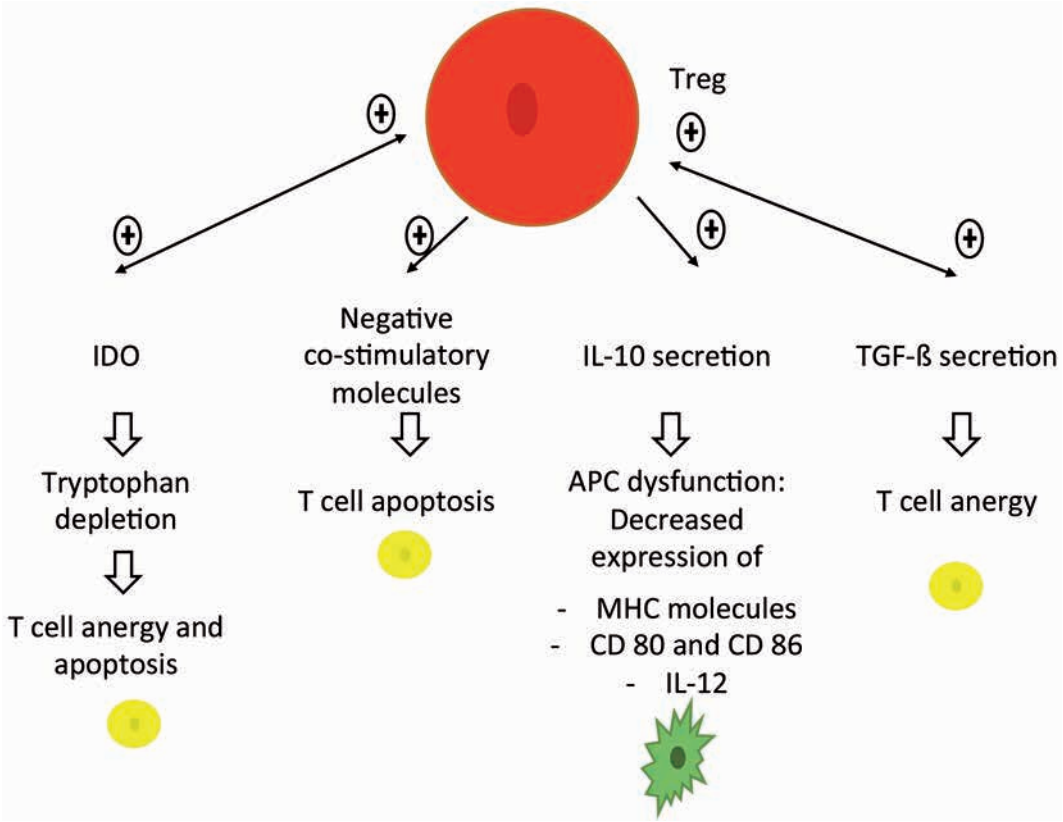

Fig. 1. — The role of regulatory T cells (Treg) in the tumor microenvironment: a schematic overview of the most important immunosuppressive mechanisms through which regulatory T cells function and the possibility of feedback-loops. Indoleamine 2,3-dioxygenase (IDO) is an intracellular enzyme that catalyses the oxidative catabolism of tryptophan. IDO suppresses T cell responses and promotes immune tolerance in mammalian pregnancy, tumor resistance, chronic infection, autoimmunity and allergic inflammation. TGF-β: transforming growth factor-β; APC: antigen presenting cell; MHC: major histocompatibility complex; CD: cluster of differentiation.

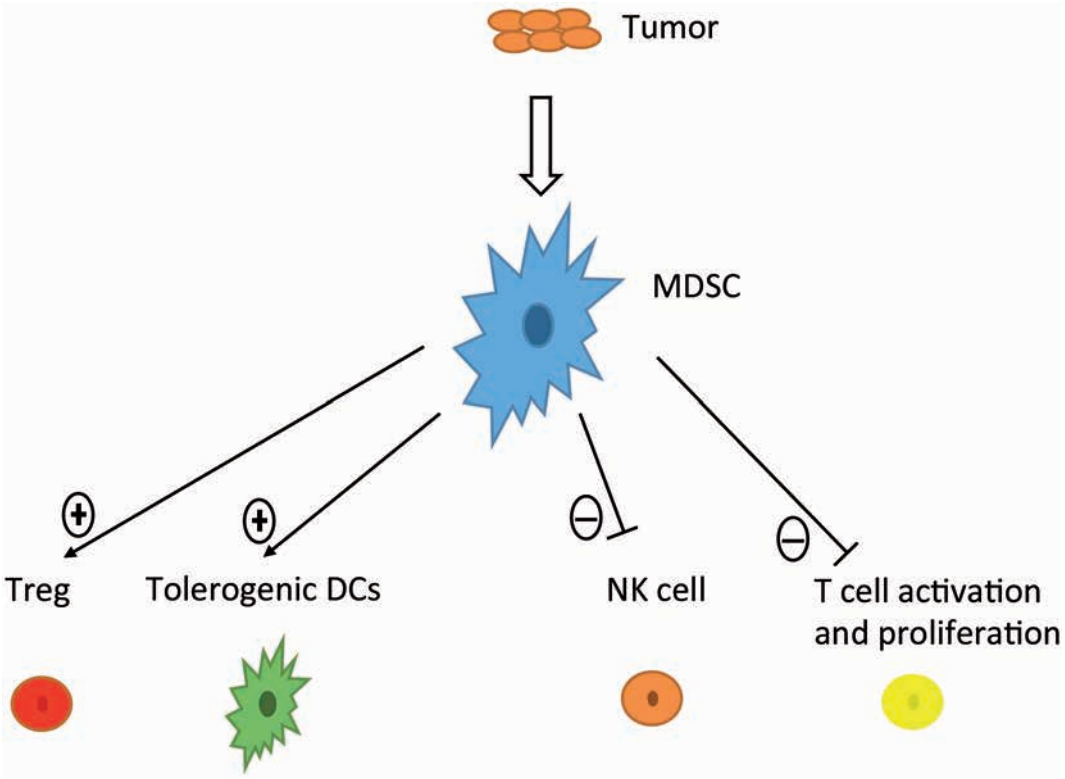

Fig. 2. — The role of Myeloid Derived Supressor Cells (MDSC) in the tumor microenvironment: an overview of the most relevant immunomodulatory functions of tumor associated myeloid derived suppressor cells stimulating immunosuppressive cells and inhibiting anti-tumor effector cells. Treg: regulatory T cell; DC: dendritic cell; NK cell: natural killer cell.

influenced by the tumor environment they will lead to tolerogenic IDO-producing DCs (Gabrilovich et al., 2012). MDSC will inhibit NK cells and cause T cell anergy through the production of TGF-β. As TGF-β is also one of the molecules that will attract MDSC this will again cause a positive feedback loop. MDSC will also inhibit the function of NK cells through interaction with their NK cell receptor NKp30. Therefore MDSC are the main negative regulator of NK cell function in a tumor-bearing host (Hoechst et al., 2009; Li et al., 2009). The main immunosuppressive role of MDSCs is the inhibition of T cell activation and proliferation. In general tumor-associated MDSC will deprive T cells of amino-acids, such as L-arginine necessary for their proliferation. MDSC will inhibit activation of the T cell through oxidation or nitrosylation of the T-cell receptor. Furthermore MDSCs will interfere with T cell migration and viability (Gabrilovich et al., 2012).

Tumor-associated macrophages (TAM) are a type of white blood cells that engulf and digest cellular debris, foreign substances, bacteria and cancer cells by phagocytosis. Macrophages play a critical role in innate immunity and help initiate the adaptive immune response by recruiting other immune cells such as lymphocytes. We can generally classify TAM in two categories: immunostimulatory M1 macrophages and immunosuppressive M2 macrophages. Once attracted to the tumor we often see a switch from M1 to M2 macrophages. Because of this switch, the role of TAM in the tumor-microenvironment is complex. The physiological role of M1 macrophages is pathogen clearance and antigen presentation to NK cells and T cells, which leads to activation of the adaptive immune system. On the other hand, M2 macrophages have a low phagocytic capacity and will not properly stimulate NK cells and T cells. They will even counteract the immune response by producing immunosuppressive molecules, leading to poor prognosis with an increased number of TAM.

Intratumoral TAM are in general immuno-suppressive and will inhibit an anti-tumoral immune response. Differentiation of monocytes into M1 macrophages is promoted by interferon-γ, lipo-polysaccharide (LPS) and other microbial products, leading to killing of bacteria, immunostimulatory functions and anti-tumor cytotoxicity (Sica et al., 2008). The major factors causing the switch from immunostimulatory M1 macrophages to immuno-suppressive M2 macrophages in the tumor micro-environment are hypoxia, macrophage colony stimulating factor-1 (CSF-1), TGF-β, IL-4, IL-13 and the presence of apoptosis (Noy et al., 2014). As shown in Figure 3 there are several mechanisms by

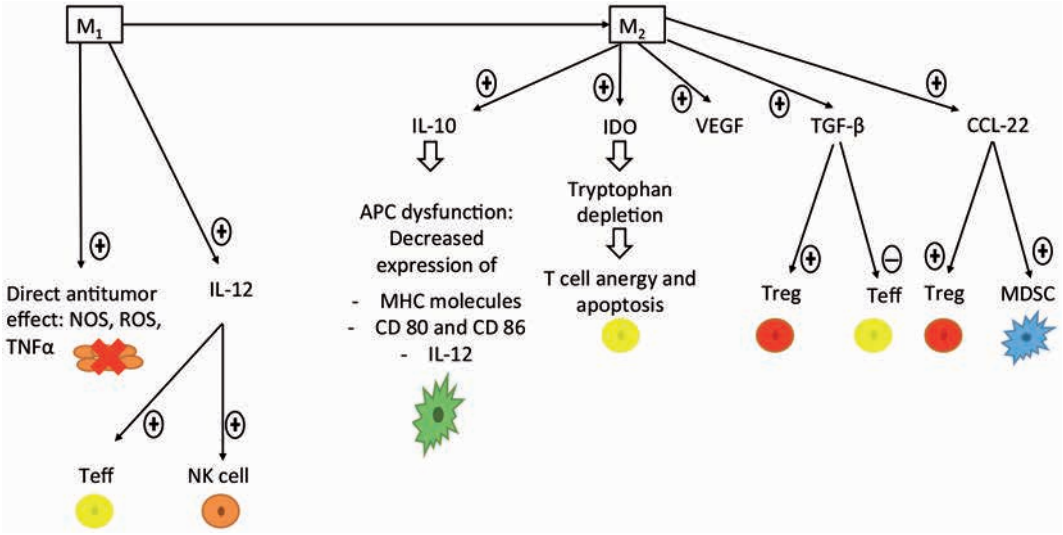

Fig. 3. — Macrophages in the tumor microenvironment. M1 macrophages are immunostimulatory cells that produce high amounts of nitric oxide (NO) and reactive oxygen intermediates to effectively destroy tumor cells. Through the L-arginine pathway M2 macrophages will inhibit the NO production of M2 macrophages and produce an anti-inflammatory response.[HYPERLINK \l "Sic08" [1] Teff: effector T cell; NK cell: natural killer cell; TGF-β: transforming growth factor-β; APC: antigen presenting cell; MHC: major histocompatibility complex; CD: cluster of differentiation; IDO: Indoleamine 2,3-dioxygenase; MDSC: myeloid derived suppressor cell; VEGF: vascular endothelial growth factor.

which M2 macrophages cause immunosuppression. M2 macrophages have shown an enhanced expression of IL-10, leading to decreased expression of IL-12 and co-stimulatory molecules by APC (Laoui et al., 2014; Ruffell et al., 2014). M2 macrophages will inhibit Teff through stimulation of IDO and increased secretion of TGF-β (Sica et al., 2008). M2 macrophages will also increase neo-vasculogenesis through increased secretion of VEGF, which conversely will also increase polarization towards M2 (Noy et al., 2014). Furthermore, M2 macrophages will also increase CCL-22, a chemokine that attracts Treg and MDSC to the tumor microenvironment (Curiel et al., 2004). These feedback-loops that strengthen the immunosuppressive microenvironment in the tumor, making it nearly impossible for Teff to significantly reduce tumor volume.

Immunosuppressive cells in ovarian cancer: situation at diagnosis

The prognostic value of immunosuppressive cells in ovarian cancer has been rarely studied. The available literature focuses merely on the presence of immune cells in the tumor rather than on their systemic presence in blood. A recent meta-analysis by Hwang et al has shown a significant survival advantage linked to tumor-infiltrating lymphocytes (TIL), however this biomarker is not yet used in clinical practice, possibly due to differences in definition of TIL (Hwang et al., 2012). Curiel et al. (2004) studied intra-tumoral Treg, which correlated with a reduced survival. PD-L1 expression on tumor cells was shown to be an independent variable correlated with poor survival (Hamanishi et al., 2007). To date no studies have been able to correlate immunosuppressive serum markers, such as TGF-β andIL-10 at diagnosis with prognosis (Mustea et al., 2009; Tas et al., 2014). A recent study correlated VEGF, survivin and second mitochondria-derived activator of caspase (Smac/DIABLO) serum levels to poor prognosis. Survivin and Smac/DIABLO are inhibitors of apoptosis (Dobrzycka et al., 2015).

Future perspectives

The discovery of the importance of the immune system in the development of cancer can help us in the determination of new diagnostic and prognostic parameters. An advantage of the determination of an immunophenotype, compared to genetic differentiation, is bifold. When mapping the immunosuppressive phenotype we do not only take into account the characteristics of the tumor, but also the environment in which the tumor grows. Clinically, this tumor environment is very relevant, as we know that patients with HIV, patients on immunosuppressive drugs against transplant rejection, … are prone to malignancies and have a worse prognosis. A second advantage is the possibility of using serum markers instead of biopsies. This evades the possibility of non-representative biopsies, inter-metastasis-heterogeneity and might be able to avoid invasive diagnostic techniques.

Treg, MDSC and TAM are detectable in blood and their secreted compounds are detectable in serum. It might thus be relevant to try to detect these cells in women with suspected ovarian masses. Just like CA125 in the ADNEX model, these cells or their metabolites might be a useful partner with added value to our existing diagnostic tools. And maybe on the long run, it will even be possible to differentiate patients into different subtypes, reflecting a different immunosuppressive signature, representing a possibility to improve outcome through tailored approaches.

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References

Curiel T, Coukos G, Zou L et al. Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. Nat Med. 2004;10:942-9.

Dobrzycka B, Mackowiak-Matejczyk B, Terlikowska KM et al. Prognostic significance of pretreatment VEGF, survivin and Smac/DIABLO serum levels in patients with serous ovarian carcinoma. Tum Biol. 2015; Epub ahead of print.

Gabrilovich D, Ostrand-Rosenberg S, Bronte V. Coordinated regulation of myeloid cells by tumours. Nat Rev Immunol. 2012;12:253-68.

Gajewski T, Schreiber H, Fu YX. Innate and adaptive immune cells in the tumor microenvironment. Nat immunol. 2013;14: 1014-22.

Galon J, Costes A, Sanchez-Carbo F et al. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science. 2006;313:1960-4.

Hamanishi J, Mandai M, Iwasaki M et al. Programmed cell death 1 ligand 1 and tumor-infiltrating CD8+ T lymphocytes are prognostic factors of human ovarian cancer. PNAS. 2007;104:3360-5.

Harlin H, Kuna T, Peterson A et al. Tumor progression despite massive influx of activated CD8+ T cells in a patient with malignant melanoma ascites. Cancer Immunol Immun. 2006;55:1185-97.

Hoechst B, Voigtlaender T, Ormandy L et al. Myeloid derived suppressor cells inhibit natural killer cells in patients with hepatocellular carcinoma via the NKp30 receptor. Hepatology. 2009;50:799-807.

Hwang WT, Adams S, Tahirovic E et al. Prognostic significance of tumor-infiltrating T cells in ovarian cancer: a meta-analysis. Gynecol Oncol. 2012;124:192-8.

Kaijser J, Vandecaveye V, Deroose C et al. Imaging techniques for the pre-surgical diagnosis of adnexal tumours. Best Pract Res Cl Ob. 2014;28:683-95.

Katz J, Muller A, Prendergast G. Indoleamine 2,3-dioxygenase in T-cell tolerance and tumoral immune escape. Immunol Rev. 2008;222:206-21.

Laoui D, Van Overmeire E, Di Conza G et al. Tumor hypoxia does not drive differentiation of tumor-associated macrophages but rather fine tunes the M2-like macrophage population.. Cancer Res. 2014;74:24-30.

Maloy K, Powrie F. Regulatory T cells in the control of immune pathology. Nat Immunol. 2001;2:816-22.

Michielsen K, Vergote I, Op de beeck K et al. Whole-body MRI with diffusion-weighted sequence for staging of patients with suspected ovarian cancer: a clinical feasibility study in comparison to CT and FDG-PET/CT. Eur Radiol. 2014;24: 889-901.

Mustea A, Braicu E, Koensgen D et al. Monitoring of IL-10 in the serum of patients with advanced ovarian cancer: results from a prospective pilot-study. Cytokine. 2009;45:8-11.

Nagaraj S, Gabrilovich D. Myeloid-Derived Supressor Cells in Human Cancer. Cancer J. 2010;16:348-53.

Noy R, Pollard J. Tumor-Associated Macrophages: From Mechanisms to Therapy. Immunity. 2014;41:49-61.

Ooi YC, Tran P, Ung N et al. The role of regulatory T-cells in glioma immunology. Clin Neurol Neurosur. 2014;119:125-32.

Rubin S, Benjamin I, Behbakht K et al. Clinical and Pathological Features of Ovarian Cancer in Women with Germ-Line Mutations of BRCA1. NEJM. 1996;335:1413-6.

Schreiber RD, Old LJ, Smyth MJ. Cancer Immunoediting: Integrating Immunity's Roles in Cancer Supression and Promotion. Science. 2011;331:1565-70.

Sica A, Larghi P, Mancino A et al. Macrophage polarization in tumour progression. Semin Cancer Biol. 2008;18:349-55.

Stephen TRM, Allegrezza M, Perales-Puchalt A et al. Transforming Growth Factor β-Mediated Suppression of Antitumor T Cells Requires FoxP1 Transcription Factor Expression. Immunity. 2014;41:427-39.

Sun C, Li N, Ding D et al. The Role of BRCA Status on the Prognosis of Patients with Epithelial Ovarian Cancer: A Systematic Review of the Literature with a Meta-Analysis. Plos One. 2014;9:e95285.

Talmadge J, Gabrilovich D. History of myeloid-derived supressor cells. Nat Rev Cancer. 2013;13:739-52.

Tas G, Karaulut S, Serilmez M et al. Clinical significance of serum transforming growth factor-beta 1 levels in patients with epithelial ovarian cancer. Tum Biol. 2014;35:3611-6.

The Cancer Genome Atlas Research Network. Integrated genomic analyses of ovarian carcinoma. Nature. 2011;474:609-15.

Van Calster B, Van Hoorde K, Valentin L et al. Evaluating the risk of ovarian cancer before surgery using the ADNEX model to differentiate between benign, borderline, early and advanced stage invasive, and secondary metastatic tumours: prospective multicentre diagnostic study. BMJ. 2014;349: g9520.

Vergote I, Tropé C, Amant F et al. Neoadjuvant chemotherapy or primary surgery in stage IIIc or IV ovarian cancer. NEJM. 2010;63:943-53.

Zhang L, Conejo-Garcia J, Katsaros D et al. Intratumoral T Cells, Recurrence, and Survival in Epithelial Ovarian Cancer. NEJM. 2003;348:203-13.

Zheng SG, Wang JH, Gray D et al. Natural and Induced CD4+CD25+ Cells Educate CD4+CD25– Cells to Develop Suppressive Activity: The Role of IL-2, TGF-β, and IL-101. J Immunol. 2004;172:5213-21.

Zou W. Regulatory T cells, tumour immunity and immunotherapy. Nat Rev Immunol. 2006;6:295-307.